ARS 44-195
November 1967

Current Status of the Avian Leukosis Complex

Agricultural Research Service
U. S. DEPARTMENT OF AGRICULTURE

CURRENT STATUS OF THE AVIAN LEUKOSIS COMPLEX 1/

B. R. Burmester 2/

During the past few years there has been a remarkable increase in significant new findings concerning the various neoplasms or diseases of the avian leukosis complex.

Undoubtedly the most important single advance is the recognition that the neoplasms that have been included in the avian leukosis complex can be divided into at least two distinct disease syndromes. The primary basis for this separation is their etiology, and this is the most important aspect of any disease.

The recognition of two distinct diseases, each causing neoplasms similar in appearance, did not come suddenly but was the result of much research over several years. However, proof came only a few weeks ago when both the Houghton Poultry Research Station in England and the U. S. Regional Poultry Research Laboratory in East Lansing, independently and at the same time, reported the recovery and demonstration of a herpesvirus as a very good candidate for the cause of Marek's disease.

This publication briefly summarizes the new information and points out notable differences and similarities of the two neoplastic diseases that are responsible for such heavy losses in the poultry industry.

TERMINOLOGY AND CLASSIFICATION

The currently used terminology and classification of the various neoplasms that have been included within the avian leukosis complex are as follows:

I. LEUKOSIS-SARCOMA GROUP--RNA, Myxovirus

Lymphoid leukosis (visceral lymphomatosis, lymphocytoma, big liver disease)

1/ Presented at the 56th Annual Meeting of the Poultry Science Association, Durham, N.H., August 21-26, 1967.

2/ Director, Regional Poultry Research Laboratory, Animal Husbandry Research Division, Agricultural Research Service, East Lansing, Mich. 48823.

Myeloblastosis (myeloid leukosis, leukemia)
Erythroblastosis (erythroleukosis)
Myelocytomosis
Fibrosarcoma, endothelioma, kidney tumor
Osteopetrosis

II. MAREK'S DISEASE--DNA, Herpesvirus

a. Classical)
b. Acute) all lymphoid (Neural, ocular, visceral (lymphomatosis, fowl paralysis, (gray eye, etc.

III. OTHERS--Unclassified

Reticuloendotheliosis

The leukosis-sarcoma group has been further classified into subgroups A, B, and C based on the host range interference patterns and into serotypes based on antigenic differences.

LEUKOSIS-SARCOMA GROUP

Physical Characteristics

The neoplasms of this group are all caused by viruses that have similar basic characteristics. All have ribonucleic acid (RNA) as the primary constituent of the core of the virus, and they have the general characteristics of the myxovirus group. Thus, the capsid is spherical with capsomeres attached to a helical coil of ribonucleic acid. The core is about 40 mμ in diameter; the entire particle, about 100 mμ in diameter.

The virus reproduces by budding from the infected cell membrane. Thus, some constituents of the virus come from host cell membrane. Virus particles may accumulate in intercellular spaces and in vacuoles but never within the nucleus or cytoplasm of the cell.

Biological Characteristics

General

For practical purposes viruses of the leukosis-sarcoma group can

be divided into (1) those that cause the various leukosis and certain solid tumors such as endotheliomas, kidney tumors, and osteopetrosis, and (2) those that cause the fibrosarcomas.

All the leukosis viruses cause either erythroblastosis or myeloblastosis at high dosages and lymphoid leukosis at lower dosages. All field isolates of virus from cases of lymphoid leukosis, when passaged rapidly, will soon cause a high incidence of erythroblastosis. Clone-isolated viruses also have invariably caused not a single tumor type but, in addition to lymphoid leukosis, have caused erythroblastosis, osteopetrosis, and endothelial tumors. Some have also caused reticular-sarcomas and fibrosarcomas. These clone-isolated virus experiments provide proof that one virus may cause a wide spectrum of different neoplasms. The relative proportion of the various tumors induced varies greatly and depends on the detailed character of the virus, the host, and the route and the dosage of virus employed.

These basic leukosis viruses can act as "helpers" to defective sarcoma viruses. Most fibrosarcoma virus strains, of which the Rous sarcoma is a prime example, are in fact mixtures of viruses--complete fibrosarcoma virus, fibrosarcoma virus genome, and a helper virus; i.e., helper virus by definition is always a leukosis virus.

The helper virus imparts four important characteristics to the fibrosarcoma virus genome. They are:

1. Infectivity. Without the helper virus, the defective or incomplete sarcoma virus cannot infect cells in vitro or in the chicken.

2. Interference among members of the same virus subgroup.

3. Host range among genetically different chickens.

4. Type specific antigenicity.

The location of these helper characters is in the envelope or outer protein coat of the virus; thus, the helper virus functions by determining the character of the outer coat of the sarcoma virus.

This basic information on the structure and function of the sarcoma viruses is very important in developing controls for lymphoid leukosis because:

1. The sarcoma viruses can be easily measured and characterized in tissue culture, in the embryo, and in the chicken.

2. Of great importance is the fact that any lymphoid leukosis virus, whether it is a recent field isolate or a long established laboratory strain, can be "married," i.e., attached to a defective sarcoma virus, with the result that we have a very useful sarcoma virus with all the important characteristics of the lymphoid leukosis virus. With confidence we can use this synthesized or so-called pseudo-Rous sarcoma virus for the following purposes:

   a. Detect infection in a flock by the RIF (resistance inducing factor) test
   b. Detect congenital transmission
   c. Test for genetic resistance to specific virus types
   d. Check for specific antibody type
   e. Develop vaccines

3. Perhaps most important is that all the research advances concerning the properties of the envelope proteins of the sarcoma virus applies completely to the lymphoid leukosis virus.

Subgrouping

The subgroup classification of the leukosis-sarcoma viruses has already been shown. The fact that this classification is based on properties of the outer coat is important.

The properties most important to us and which go hand in hand in defining the subgroups are (1) the host range, (2) the interference patterns, and (3) the primary antigenic types.

Host range--In general the genetic-determined resistance to lymphoid leukosis operates at two, and perhaps three, distinct levels. One is at the level of the cell and determines whether or not the cell becomes infected with the virus. This has been referred to as cellular resistance. The second operates at the level of the entire chicken and determines whether or not a tumor results from the infection.

There is some indication of a third type that has a marked effect on the regression of tumors and on the recovery and survival of the chicken. In other words, a chicken may or may not become infected, an infected chicken may or may not develop tumors, and a chicken with a tumor may or may not die with a progressively growing tumor. It appears that the genetic factors for these three types of resistance are entirely different from each other.

With respect to the cellular resistance and susceptibility, it is apparent that whether or not a cell becomes infected with one of these viruses depends on two interrelated factors. They are the character of the virus coat and the character of the cell surface. It appears that specific cell receptors must be present to provide for attachment and penetration of the virus. These cell surface receptors are specific and will attach only viruses having a protein coat of specific type.

In testing many different lines of chickens, embryos, or cultured cells with viruses representing the three subgroups, six different phenotypes have been identified (table 1).

A partial list of the tumor virus strains that have been classified into the virus subgroups A, B, and C is shown in table 2. The strains have been grouped into leukosis viruses, Rous sarcoma viruses (RSV), and sarcoma pseudotype viruses. Please note that many sarcoma and some leukosis strains consist of more than one subgroup virus. Thus, Schmidt-Ruppin has subgroup A and B viruses, and both the Carr-Zilber and Prague are a mixture of subgroups A, B, and C. These have been separated by cell culture procedures. Also note that 5 different leukosis viruses designated RAV have been isolated from the Bryan standard strain of RSV. All but one of these RAV-2, are subgroup A, but they are all serologically distinct.

When a specific leukosis virus is attached to a defective RSV, a complete pseudotype virus results. The variety possible is dependent on the number of distinct leukosis strains available.

Interference--The property of interference has been used extensively in assaying for the leukosis virus and also in delineating the virus subgroups. Interference is the basis for the RIF test. When genetically susceptible chick embryo fibroblasts are seeded with Rous virus, foci of altered cells are induced. The number of foci is directionally proportional to the inoculation dose. However, if the cells are first infected, either naturally via the infected egg or are seeded with a leukosis virus, they become resistant to foci formation with RSV. This resistance is not absolute and occurs only when the leukosis virus is given a "head start" over the RSV. In addition, interference occurs only between viruses having a similar protein coat. Thus, leukosis virus of subgroup A will interfere with RSV of subgroup A but will not interfere with RSV of subgroup B or C.

Antigenic specificity--Differences between virus strains, groups, or types can be detected with greatest sensitivity by the use of antibody neutralizations and fluorescent staining methods.

Table 1.--Response of various host phenotypes to three subgroups of leukosis-sarcoma viruses

Host Phenotypes 1/	Virus Subgroups 2/		
	A	B	C
C/A	O	T	T
C/B	T	O	T
C/C	T	T	O
C/O	T	T	T
C/AB	O	O	T
C/AC	O	T	O
C/BC	T	O	O
C/ABC	O	O	O

1/ Phenotypes not underlined have not yet been found. Symbols have the following meaning: C, chicken; /, resistant to; A, B, and C, virus subgroup.

2/ Symbols have the following meaning: T, tumor; O, no response.

Table 2.--Partial list of tumor virus strains and subgroup classification

Virus strain	Virus subgroups and designation		
	A	B	C
Leukosis			
Lymphomatosis-erythroblastosis-	RPL-12-1		
Myeloblastosis BAI-A	AMV-1	AMV-2	
RSV associated (St. Strain)	RAV-1, RAV-3, RAV-4, RAV-5	RAV-2	RAV-50.
Fujinami associated	FAV-1	FAV-2	
Rous Sarcoma			
Bryan Standard	BS-RSV-1		
Schmidt-Ruppin	SR-RSV-1	SR-RSV-2	
Harris		HA-RSV	
Carr-Zilber	CZ-RSV-1	CZ-RSV-2	CZ-RSV-3.
Prague	PR-RSV-1	PR-RSV-2	PR-RSV-3.
Sarcoma Pseudotype			
RSV-Bryan (H-T)	BH-RSV(RAV-1)	BH-RSV(RAV-2)	
	BH-RSV(RAV-3)		
	BH-RSV(AMV-1)	BH-RSV(AMV-2)	
	BH-RSV(RPL-12)		
Fujinami Standard Strain	FSV(FAV-1)	FSV(FAV-2)	

As given previously, the primary serotypes fall into the same groups as those specified by the host range and interference patterns. This is expected because all three characters are dependent on the outer coat of the virus. In addition, it has been found that most viral strains can be differentiated serologically by quantitative differences in the reaction between antigen and antibody.

Control Program

Eradication

Infection caused by the leukosis-sarcoma viruses has been eradicated from a number of experimental and special-purpose flocks. This has been done by use of an expensive testing program, segregation, and isolation rearing and breeding. Experience has shown that once the infection has been eliminated, extreme measures are not required to keep out reinfection. Whether or not eradication is a practical procedure for commercial poultry depends largely on three factors: The cost of the testing program, the infection rate, and the possibility of reinfection.

A number of tests have been developed that are useful for the detection of the virus or antibody of lymphoid leukosis. Most of these tests depend on a tissue culture system and are relatively expensive to conduct.

RIF test--The first available laboratory test for lymphoid leukosis virus is the RIF test. It was used to establish the present RIF-free flocks. The basis for this test has already been described. Its primary drawbacks are (a) the long period required for the test, (b) chick embryo fibroblasts must be maintained in excellent physiological condition for long periods, and (c) because interference is based on properties of the outer coat, a test virus appropriate for each of the three subgroups must be employed.

COFAL test--This is a complement fixation test and is dependent on an antigen of the nucleus or core of the virus stimulating a respective antibody in a mammal. The hamster and the Schmidt-Ruppin strain of RSV are most often used. The hamster antiserum is then used in the complement fixation test to detect the same nucleoid virus antigen in the chicken to be tested. Because a single type of nucleoid is common to all viruses of the leukosis-sarcoma group, only one set of reagents is needed to detect all infections of this subgroup.

NP test--The basis of this test is the NP or nonproducer cells. Cells that have been transformed into neoplastic cells by the incomplete or defective RSV do not produce infectious virus. However, when they are grown with cells that are infected with a leukosis virus, the defective RSV becomes complete and infectious virus is replicated. The complete virus is then easily detected by the inoculation of cell cultures, embryos, or chickens.

FA test--An indirect fluorescent antibody test has been developed that detects viral antigen in chicken fibroblast cultures. This test has been found highly useful for the detection of serum antibodies and of virus in sera or in embryos. It is subgroup specific; thus, reagents of each subgroup must be used.

Neutralization test--This test has been used extensively for an indication of current and past infection and has proved highly useful for the monitoring of RIF-free flocks for possible recontamination. Since the test depends on the outer coat of the virus, viruses representative of each subgroup in question must be employed in the test.

Genetic Resistance

Breeding for resistance to infection is currently a good possibility for control. It has been well established that a single autosomal recessive gene controls specific cellular resistance to the in vitro or in vivo growth of subgroup A viruses. Similarly, a second autosomal recessive gene provides resistance to subgroup B virus. Less is known of the inheritance of resistance to subgroup C virus. Preliminary data indicate that it is similarly controlled by a third recessive gene. The genes for A and B viruses and probably the C viruses have been found to segregate independently, and they are not sex linked. However, there is evidence that under certain conditions phenotypic mixing between viruses of different subgroups can occur.

Because resistance to infection is subgroup specific, information on prevalence of infection with the various subgroup viruses and of resistant genes is of much interest. Limited data indicate that in commercial flocks infection with subgroup C viruses is extremely rare; infection with subgroup B viruses occurs sporadically; and infection with subgroup A viruses is by far the most prevalent. Generally the rate of congenital infection is 5 to 10 percent. By the time the chickens are sexually mature, however, the infection generally has spread to 50-95 percent of the chickens.

Of equal interest is the occurrence of resistance genes in commercial breeds and strains of chickens. Preliminary surveys have shown that subgroup A resistance occurs at a low rate in chickens of egg-laying breeds, strains, or crosses, but some flocks have a level that will allow a reasonable rate of increase by suitable selection. The genes for resistance to subgroup A viruses in heavy breeds and for subgroup B viruses in all breeds are much more common. No information is available on the occurrence of subgroup C resistance in commercial stocks.

The foregoing observations are in agreement with the logical assumption that with an increase in percentage of individuals resistant to specific virus infection, such infections naturally die out. Eradication probably will have occurred before 100 percent resistance has been achieved.

Although breeding for resistance to infection has many advantages, important obstacles may arise, and breeders should not overlook the possibility of breeding for resistance to the development of neoplasms. 1/

Abalation of the Bursa of Fabricius

Studies have shown that the development of lymphoid leukosis neoplasms is dependent on the presence of a functional bursa of Fabricius. If this organ is removed, either surgically or by the use of such hormones as androgens and cortisones, tumor development is very markedly reduced or completely prevented. It is quite possible that the removal of this bursa of Fabricius may have practical application in the control of lymphoid leukosis neoplasms. However, it has no effect on the infection cycle of the disease.

Vaccines and chemotherapeutics

No vaccines have been developed or chemicals found that are effective in the prevention or treatment.

MAREK'S DISEASE GROUP

Physical Characteristics

The candidate virus for Marek's disease is a Herpesvirus. There are some 20 viruses that belong to this Herpesvirus group. They cause infections

---

1/ Crittenden, Lyman B., and Vogt, Peter K. The avian tumor viruses: Prospects for control. World's Poultry Sci. Jour. (submitted for publication)

in man, other mammals, and birds. Generally, the viruses of this group have a nucleoid or core made up of desoxyribonucleic acid (DNA); the capsid is cubic, icosahedral in symmetry and in shape, and has 162 capsomeres. Normally an envelope surrounds the capsid, and it is ether, heat and pH sensitive.

The virus found in Marek's disease (MD) cultures has a nucleoid 75 mμ in diameter, the capsid is about 130 mμ, and the entire virus, which is only seldom seen, has been estimated to be about 250 mμ in diameter.

Biological Characteristics

Information on the biological characteristics of the virus is very limited. Because the infectious virus is extremely cell associated, at least under experimental conditions, it has so far not been possible to describe many of its characters. In fact, practically all studies on

Marek's disease have been conducted with cell-containing inoculum. In spite of this, there is good indication that for most transmissions the induced tumor originates from recipient cells and not of the cells of origin; thus it is agent-induced, rather than a transplant. However, there are some examples where "hot" strains have acquired the ability of the tumor to transplant into heterologous hosts; thus, one must be cautious in making interpretations when cellular inoculum is used. In view of the foregoing, the statements to follow regarding isolates or strains of MD must be taken with some reservations.

The following information was obtained with such MD isolates as JM, GA, CONN-A, HPRS-14, and HPRS-16:

1. Only recently have investigators been able to grow MD virus in cell cultures. Despite this, it is RIF and COFAL negative. These latter are prominent features of the leukosis-sarcoma virus.

2. Infectivity is destroyed at pH of 5.5 and below, at 8.4 and above, and when held at a temperature of 56° C for 30 minutes.

3. All treatments that eliminate or destroy the integrity of the cells have reduced or eliminated the potency of the inoculum. This includes (a) filtration, (b) appropriate centrifugation, (c) freezing and thawing, (d) use of a blender or ultrasonic disintegrator, and (e) lyophilization. Results have been uniform for blood, tumor suspensions, and cell cultures, with the exception that plasma of GA isolates has remained infectious after two cycles of sedimentation and resuspension with the aid of a centrifuge.

The property of close cell association of infectivity has been encountered in working with cell cultures. The infectivity survives for variable periods in cultures of chicken -- whole embryos and of liver, spleen, bone marrow, and lung tissue. Such cultures, as well as intact chicken embryos, have not shown any alterations, and the virus appears to remain in a latent state.

Recently, scientists at the Houghton Poultry Research Station, England, reported CPE (cytopathogenic effect) in chicken kidney cultures seeded with Marek's blood. They showed that the CPE was typical of Herpesvirus and demonstrated a Herpesvirus in the cells of the culture. Furthermore, these scientists could reproduce the disease with cultures containing CPE, and not with similar cultures without CPE. Similar findings were reported at the same time by scientists of the U. S. Regional Poultry Research Laboratory in East Lansing, with the exception that the results were obtained with duck embryo cultures.

The CPE was observed in duck embryo fibroblast cultures 11 to 25 days post inoculation. All cultures that showed CPE contained Herpesvirus particles, and when chicks were inoculated, caused typical Marek's disease.

The virus particles were found in the nucleus and cytoplasm of infected cells and in extracellular material. However, complete particles with the protein envelope essential for infectivity of Herpesviruses were not found.

Although preliminary, these findings represent a major "breakthrough" in research on Marek's disease. However, many more "hurdles" are still before us. Thus, despite the fact that we can with the aid of the electron microscope see the virus particle in the nucleus, in the cytoplasm, and in extracellular spaces, we have not been able to transmit infection without cells. The reasons for this may be due to the fact that almost all virus particles that have been seen in these cultures are without the outer envelope. Cellular requirement for infectivity and lack of outer envelope are characteristics similar to those of the Herpesviruses of subgroup B, such as Herpes zoster of man, and the cytomegaloviruses of man and various other mammals, all of which are extremely cell-associated. At the same time, under certain natural conditions, they are highly contagious. Marek's disease is also highly contagious. It is a common observation that MD readily spreads horizontally from flock to flock. In addition, controlled experiments have shown high rates of transmission via the air. Also, droppings at room temperature have remained infectious for long periods.

Control Program

Classical Marek's disease has been observed since the turn of the century and now occurs to a limited extent in the poultry of all sections of the United States. Acute Marek's disease in growing stock is of much more recent origin and has been more restricted in location. However, acute Marek's disease has spread to new areas and currently its absence in any section with commercial poultry is questionable.

Eradication

No laboratory methods useful in an eradication program are currently available. Assay for the Marek's disease virus is now confined to the inoculation of susceptible chicks, keeping them in Horsfall-Bauer type isolators, and then measuring their responses in 3 to 8 weeks. Some analysis can be done with the electron microscope. However, this is limited to specific cell cultures because many highly infectious materials have not revealed overt Herpes particles.

The cell culture holds promise for a possible in vitro test method. However, many advances must be made before it is to be a useful detection or assay procedure.

A fluorescent antibody test also appears to hold some promise. Here again, much needs to be accomplished before this test can be used as a routine method.

Genetic Resistance

Genetics-determined resistance to acute Marek's disease has been well demonstrated. In only two generations of selection on the basis of the response of progeny to inoculation with the JM strain of Marek's disease, one investigator segregated a resistant line with 13 percent MD and a susceptible line with 91 percent MD from the original unselected breeders, which had 51 percent MD mortality after inoculation. Much more extensive experience with commercial strains and crosses will be required to determine whether this promising procedure will result in a practical control of the disease.

Vaccines and Chemotherapeutics

Many investigators and poultrymen have observed the apparent recovery of paralyzed or otherwise affected birds with Marek's disease. However, we have as yet no direct evidence for an immune response nor the occurrence of antibodies in exposed chickens. Neutralization tests have given negative results. This may be due to the virus being cell-contained and not accessible to antibodies. The isolation and identification of the causative virus are major steps toward investigation of the immune response and the possible development of a vaccine.

No chemical agent has been identified that is in any way effective against Marek's disease.

DIFFERENTIAL DIAGNOSIS

Until about 2 years ago, Marek's disease was included under lymphomatosis -- neural, visceral, or ocular. No thought was given to differential diagnosis because (a) the preceding term was a lesion or a pathologic diagnosis, and (b) there was no conclusive evidence that two different diseases were placed in the same "bucket." Now that it has been proved two distinct diseases cause similar lesions and that appropriate changes in nomenclature have been made, the problem of differential diagnosis has to be faced.

Generally, the diagnosis should be based on the examination of several live but typically affected birds and a consideration of the flock history. In some flocks beyond 4 months of age, mortality may result from neoplasms caused by both diseases. Obviously, such flocks present a confusing picture, and differential diagnosis must be attempted on the basis of individual bird examinations.

The following differential features are available to the diagnostician: (LL = lymphoid leukosis; MD = Marek's disease).

1. Occurrence

LL usually has a low continuous rate with a moderate to low peak at sexual maturity.

MD (a) acute has moderate to high rate with a distinct peak; and

MD (b) classical has low to moderate rate with a moderate peak prior to sexual maturity.

2. Age

LL does not occur in less than 4 months, usually 5 to 6 months.

MD usually occurs at 1 to 4 months but often after this time.

3. Lesion location

LL lesions are never in the nerve; almost always in the bursa of Fabricius, but they may be quite small; usually in the liver and spleen, and often in other viscera.

MD lesions are very rarely in the bursa, usually in the nerves; often gonad and many others.

4. Gross pathology

Not distinctive.

5. Micropathology

When typical lesions are not found in the nervous system or when mixed lesions are suspected, a cytologic examination of the visceral lesion is appropriate and helpful in making a differential diagnosis.

LL generally has a uniform population of lymphoblastic cells and large lymphocytes. The nucleolus is prominent, and the chromatin is diffuse.

MD the lesion generally consists of all forms of the lymphocytic series. The nucleolus is present only in the large lymphocytes and blast cells, and then it is not as prominent as in LL.

These differential features can best be seen when smears or touch preparations of the lesion are made and Shorr's stain or Methyl Green Pyronin stain is employed. With the latter stain, the cytoplasm and nucleolus of the immature cells such as the lymphoblast stain a bright red, in contrast to the mature cells that stain a pale blue.

SUMMARY

Two distinct diseases that cause either high death rates or condemnation due to lymphoid tumors in various tissues of the chicken are now recognized. One is now referred to as lymphoid leukosis and is caused by an RNA virus, and three virus subgroups have been identified. It is commonly egg-transmitted but is not highly contagious. Infection, or tumor induction, or both are genetically conditioned. Tumor formation requires a functional bursa of Fabricius.

The second disease is referred to as Marek's disease. It also is a disease of the lymphoid series cells but is caused by a DNA virus of the Herpes group. It is highly contagious, and the significance of egg transmission is debatable. Induction of disease is also genetically conditioned.

SELECTED REFERENCES

Biggs, P. M., and Payne, L. M.

1967. The avian leukosis complex. Vet. Rec. 80: 5-7.

Burmester, B. R.

1962. The vertical and horizontal transmission of avian lymphomatosis.

Cold Spring Harbor Symposia on Quantitative Biology 27: 471-477.

Burmester, B. R., and Witter, R. L.

1966. An outline of the avian leukosis complex. U. S. Dept. Agr.

Prod. Res. Rpt. 94, 8 pp.

Vogt, Peter.

1965. Avian tumor viruses. Advances in Virus Res. 11: 294-374.

Academic Press, New York and London.